Reviews in Toxicology 1 (1997) 33–51
Biochemical Toxicology
IOS Press

The importance of enzymatic biotransformation in immunotoxicology

Dori R. Germolec[1*], Nancy H. Adams[2] and Michael I. Luster[3]
[1]Environmental Immunology Group, National Institute of Environmental Health Sciences, Research Triangle Park, NC., 27709, USA; [2]United States Environmental Protection Agency, Research Triangle Park, NC, 27709, USA; [3]National Institute of Occupational Safety and Health, Morgantown, WVa, 26505, USA

Abstract. Many immunotoxic compounds, such as benzene and other organic solvents, pesticides, mycotoxins and polycyclic aromatic hydrocarbons, can alter immune function only after undergoing enzyme-mediated reactions within various tissues. In the review that follows the role of enzymatic transformation in immunotoxicity is examined. We begin with a brief overview of the immune system and a summary of the evidence which suggests that xenobiotics can alter the function of the cells and signaling molecules required for normal immune responses. We then examine the principal Phase I and Phase II enzymes involved in the bioactivation process, particularly the cytochrome P450s, the reactions by which these enzymes detoxify or bioactivate foreign compounds, and the factors which influence their expression and regulation. Finally, we present a number of immunotoxicants and discuss the role that metabolic activation plays in their toxicity.

1. The immune system and immunosuppression

The immune system is a complex set of soluble protein, cellular, and chemical components designed to protect the body against foreign substances, including infectious agents and tumor cells, while not responding to self-antigens. The distinction between self and nonself is made by a complex system that depends upon specific recognition molecules present on the surface of T and B lymphocytes. Nonspecific effector mechanisms that complement or amplify the specific T and B lymphocyte responses are also important in the immune response. These nonspecific entities serve as a first line of defense against potential pathogens and include other leukocytes such as macrophages, natural killer (NK) cells, polymorphonuclear (PMN) leukocytes, as well as soluble mediators such as complement and cytokines. Many of these cell populations (e.g., B lymphocytes, T lymphocytes, NK cells) can be further divided into subpopulations based on varying functional properties or states of differentiation, maturation and activation. Most notable among these are the T cell subpopulations which include cells which amplify other immune respons-

* Corresponding Author: Dora R. Germolec, NIEHS (MD C1-04), P.O. Box 12233, Research Triangle Park, NC, 27709, USA. Tel.: +1 919 541 3230; Fax: +1 919 541 0870.

1382-6980/97/$8.00

es (T-helper cells), down-regulate other immune responses (T-suppressor cells) and destroy viral or tumor-infected cells (cytotoxic T cells).

Immune cells are located throughout the body, either in discretely encapsulated organs, such as the spleen and thymus, or as diffuse accumulations of lymphoid and myeloid cells, such as are found in association with the skin and gut, where they are strategically placed to monitor the entry of foreign substances. Optimal function of the immune system requires that these specific cells and cell products interact with each other in a sequential, regulated manner. In addition to the complex cell-cell and cytokine interactions which are necessary in the normal functioning of the immune system, secondary factors can influence immune status, including nutritional state, neurohormones and stress.

A large body of evidence has accumulated in the past 15 years demonstrating that certain chemicals can produce immunosuppression and alter host resistance in experimental animals following acute or subchronic exposure [17,81,113]. Several studies describing similar effects in humans following either occupational or accidental exposure have been reported [70]. For the most part, these clinical studies have been criticized for their incomplete or inconsistent diagnosis of immunodeficiency as well as for a lack of clinical changes. Within the scientific community, a consensus exists that further clinical studies, using well-defined cohorts, will be required to determine whether low-level, chronic exposure to chemicals affects human health via alterations in the immune system.

Results from animal studies have shown that exposure to certain xenobiotics can produce immune dysfunction often characterized by decreased responsiveness to antigen stimulation and resistance to challenge with infectious agents or tumor cells. These studies have suggested that the immune system is a sensitive target for the toxicity of certain chemicals by showing alterations at chemical doses below those that affect other organs or systems. These chemicals may include products or byproducts from pharmaceuticals, farming, manufacturing, consumer products, food additives, or natural products such as mycotoxins.

In addition to those chemicals that cause direct damage to immune cells and tissues, there are many compounds that can impair immune function only after undergoing enzyme-mediated reactions within various tissues (Table 1). The metabolic transformations that have evolved to break down exogenous chemicals generally make these compounds less toxic (deactivation reactions) and more water-soluble, thus facilitating their elimination from the body. However, some of these compounds are transformed to active or more toxic metabolites and such reactions are referred to as activation or bioactivation reactions. Bioactivation reactions resulting in toxicity are frequently due to the formation of reactive intermediates, including epoxides, free radicals, or N-hydroxyl derivatives [36,91].

Table 1
Examples of immunotoxic compounds requiring metabolic activation

Class	Compounds
Miscellaneous	Cyclophospamide
	Dimethylnitrosamine
Mycotoxins	Aflatoxin
	Ochratoxin A
	Wortmannin
Organic Solvents	Benzene
	Carbon Tetrachloride
	Ethanol
	n-hexane
PAHs	Benzo(a)pyrene
	Dimethylbenzanthracene
	3-Methylcholanthrene
Pesticides	Chlordane
	Malathion
	Parathion

Adapted from [17,113]

2. The role of enzymatic transformation in immunotoxicity

Xenobiotic metabolism occurs via enzymatic reactions which can be broadly classified into two categories: Phase I and Phase II reactions. These reactions generally work in concert to detoxify and remove xenobiotics from the body. Phase I reactions are often oxidations, although they may also involve reductions, hydrations, ester hydrolysis, alcohol and aldehyde dehydrogenation, and superoxide dismutase reactions. The class of reactions termed Phase II generally act through conjugation of the xenobiotic molecule with a polar compound, to further increase water-solubility and accelerate excretion. Phase II reactions include methylations, acetylations, and conjugation reactions with glucuronides, sulfates, glutathione, glucose, thiols, and thiosulfates. The substrates for Phase II reactions can be the unchanged xenobiotic or the metabolic product of a Phase I reaction [91]. Table 2 lists the principal Phase I and Phase II enzymes responsible for xenobiotic metabolism.

3. Phase I metabolism and the cytochrome P450 enzymes

Although deactivation reactions predominate, the principal Phase I enzymes involved in metabolic activation are the cytochrome P450s. These enzymes are widespread in nature, found in microorganisms, plants and animals. The P450 isozymes

Table 2
Phase I and Phase II metabolic enzymes

Class	Reaction	Enzyme
Phase I Enzymes	Hydrolysis	Carboxylesterase
		Peptidase
		Epoxide hydrolase
	Reduction	Azo-, nitro-reductase
		Carbonyl reductase
		Disulfide reductase
		Sulfoxide reductase
		Quninone reductase
		Cytochrome P450
		(reductive dehalogenation)
	Oxidation	Alcohol dehydrogenase
		Aldehyde dehydrogenase
		Xanthine oxidase
		Monoamine oxidase
		Diamine oxidase
		Prostaglandin H synthase
		Flavin monooxygenase
		Cytochrome P450
Phase II Enzymes	Conjugation	UDP-glucuronosyltransferase
		Sulfotransferase
		Glutathione S-transferase
		Acyl CoA synthetase/
		N-acyltransferase
	Addition of Functional Groups	*N*-acetyltransferase
		Methyltransferase

Adapted from [91].

in the portals of entry to the body (skin, respiratory system, digestive system) are thought to act as a first line of defense by detoxifying xenobiotics before toxic insult can occur. The P450s are classified as monooxygenases because one atom of molecular oxygen is added to the substrate molecule and one atom of oxygen forms water. The P450 protein contains a single heme moiety that binds to molecular oxygen and a hydrophobic substrate-binding site [56]. The general reaction catalyzed by the P450s is characterized by the addition of a hydroxyl group to a substrate molecule:

$$RH + O_2 + NADPH + H^+ \rightarrow ROH + H_2O + NADP^+$$

The P450 system requires NADPH-cytochrome P450 reductase and lipid cofactors. Table 3 lists some of the major types of activation reactions catalyzed by P450s.

To date over 480 P450 genes have been characterized and classified into 74 gene families, fourteen of which are found in mammalian species [87]. More than twenty

Table 3
Major bioactivation reactions catalyzed by cytochrome P-450 isozymes

Type of reaction	Substrate
Aliphatic hydroxylation	n-Hexane
Aromatic hydroxylation	Cyclophosphamide, benzene
Epoxidation	Benzo(a)pyrene, AFB_1
Dealkylation	Phenacetin
Reductive dehalogenation	Carbon tetrachloride
Benzodioxole ring cleavage	Piperonyl butoxide
N-hydroxylation	2-Acetylaminfluorene

Adapted from [17,113]

distinct human isozymes are known [30]. Classification is based on amino acid sequence homology, with members of the same family exhibiting at least 40% homology, and members of the same subfamily exhibiting at least 55% homology. Individual genes and their enzyme products, for all species except the mouse and the fruit fly, are named by the root CYP, followed by an arabic number (family), a capital letter (subfamily), and a number (for the distinct gene). The corresponding mouse and fruit fly genes are named by the root, Cyp (e.g., Cyp2a4)[87]. As a general rule, all genes in a family show the same number of exons and have similar intron-exon boundaries. Although predominantly hepatic in mammals, the enzymes have been found in all tissues examined, intracellularly localized primarily to the endoplasmic reticulum and the mitochondria [97]. For certain subfamilies no exact orthologs are known across species. As a consequence, considerable species differences may exist in isozyme expression and substrate specificity within a given subfamily [33]. For example, in rats, enzymes in the CYP2C subfamily contribute to steroid hydroxylation, and are expressed in a sex-specific manner. In the rabbit, none of the 2C isozymes are expressed in a sex-specific manner, and the substrate targets are different. In humans, there are four members of the 2C subfamily which do not appear to have exact orthologs in other species. There is also no evidence of sex-specific expression of the 2C isozymes in humans, and these enzymes have little steroid hydroxylase activity [33].

Several P450 isoforms are highly specific for endogenous substrates such as steroids, bile pigments, prostaglandins, leukotrienes, biogenic amines, and fatty acids. The steroidogenic and cholesterol-metabolizing P450s in families 7,17,19, 21 and 27 are highly conserved, exhibit relatively strict substrate and product specificity, and are thought to have arisen earliest in the evolution of the enzyme system [33]. These constitutive P450 isozymes are thought to be important in maintaining homeostasis with regard to growth, differentiation, and neuroendocrine function.

Three gene families (*CYP1*, *CYP2* and *CYP3*) appear to be primarily responsible for the oxidation of foreign compounds including drugs, pesticides and environmental contaminants [38]. These families may have evolved from steroidogenic

P450s, responsible for the oxidation of endogenous compounds, to detoxify xenobiotics such as plant toxins taken in through the diet. Of the xenobiotic metabolizing P450s, CYP1A1 is the most highly conserved. An ortholog of 1A with similar catalytic properties and consistent substrate preferences is found in diverse species such as rodents, chickens and fish [83].

Table 4
Inducers of some cytochrome p-450 isozymes that catalyze bioactivation reactions

Compound/Class	Example	P-450 induced
Polycyclic aromatic hydrocarbons	3MC, PCBs	1A1, 1A2, 2A1
Barbiturates	Phenobarbital	2B1, 2B2, 2A
Chlorinated dioxins	2,3,7,8-TCDD	1A1, 1A2
Low mol. wt. organic solvents	Ethanol	2E1
Methylenedioxyphenyl cmpds.	Isosafrole	1A2
Hypolipidemic drugs	Clofibrate	4A1
Antifungals	Clotrimazole	3A

Adapted from [91].

The regulation of P450s have been studied extensively. Factors affecting P450 expression include gender, age, nutritional status, disease, genetic predeterminants, environmental pollutants, and stress [32,50,62,90]. A number of P450 isozymes have been shown to be polymorphic in humans, and null polymorphisms which result in absence of proteins (e.g. gene deletions, splicing defects) as well as more subtle alterations, such as allelic variants with slightly altered catalytic activity, have been shown in several species [75,87,88,90]. It is notable that both very young and very old organisms are deficient in many of the constitutive P450 enzymes, although certain P450 isozymes can be induced in these groups by exposure to xenobiotics. Thus, compounds can be more or less toxic as a result of age or nutritional status. Tissue concentrations of various P450s can also be influenced by a large number of lipophilic xenobiotics. Table 4 lists some known inducers of P450s and the isozymes that these compounds induce. 3-Methylcholanthrene (3MC) and phenobarbital (PB) have been widely studied as inducers of the CYP1A and the CYP2B families, respectively. Many polycyclic aromatic hydrocarbons (PAHs) have been found to induce the 1A and 1B families, with the strongest inducers found among the most planar PAHs. PAHs are metabolized by CYP1A1 and CYP1B1, a constitutive from that is regulated by steroid and peptide hormones [63]. Both 1A1 and 1B1 are under Ah receptor control [1]. Since the 1A and 1B families are involved in bioactivation reactions, inducers of these P450 families are of special concern with regard to immunotoxicity [46,63]. Another widely investigated inducer is ethanol, which induces the metabolism of low molecular weight organic solvents. Other inducers include halogenated pesticides, polychlorinated biphenyls and chlorinated dioxins [91]. Steroids such as pregnenolone-16a-carbonitrile appear to induce the

3A family preferentially. Hypolipidemic agents, such as clofibrate, induce CYP3A4. Induction is often tissue specific, and compounds that induce P450s are often, but not always, the substrates of these same P450s. Induction is generally rapid and transient, but the degree, onset, and duration of induction vary with the inducing compound and dose. Additionally, induction of one P450 isozyme may be accompanied by decreased expression of others, further perturbing the metabolic response of the organism. The expression of P450 isoforms is tissue-specific as well as substrate-specific and is regulated by a variety of mechanisms including mRNA stabilization, transcriptional rate and enzyme stabilization [97].

4. Immunotoxic compounds requiring metabolic activation

Many immunotoxic compounds, including organic solvents such as benzene, cytoreductive drugs, pesticides, mycotoxins and PAHs, require metabolic activation by Phase I enzymes in order for their toxicity to be manifested. A number of studies indicate that extrahepatic metabolism may also be involved in targeting of chemical-induced toxic effects [7,39,84]. A detailed examination of the bioactivation and immunotoxicity of several of these classes of compounds follows.

4.1. Polycyclic Aromatic Hydrocarbons

Perhaps the most extensively studied class of compounds which require metabolic activation for the induction of immunotoxicity are PAHs. PAHs make up a family of ubiquitous environmental contaminants which are metabolized to reactive electrophilic intermediates by cytochrome P450 [25]. These reactive metabolites, primarily diol-epoxides, alter normal cellular function by binding covalently to RNA, DNA and proteins. As discussed above, the CYP1A1 and CYP1B1 families are the principal enzymes involved in PAH activation. Studies in laboratory animals suggest that there is a significant correlation between suppression of antibody forming cell responses and the carcinogenic activity of PAHs [121]. Several mechanisms have been proposed to explain PAH-induced immunosuppression, including membrane perturbation resulting in altered signal transduction, calcium mobilization, gene expression and/or cytokine production [16,71,89].

In rodents, *in vivo* exposure to B(a)P inhibits both humoral- and cell-mediated immunity, as well as some aspects of innate immunity including macrophage phagocytosis and interferon production [rev. in 14,57]. *In vivo* exposure has been shown to target both primary and secondary immune tissues, and to significantly alter lymphoid cell numbers and cell surface antigen expression in the spleen, thymus and bone marrow [44]. B(a)P is metabolized primarily in the liver, and reactive metabolites are transported by serum proteins to the spleen and other tissues [28]. While DNA adduct formation is similar in spleen, lung, liver, kidney after *in vivo* exposure, cultures of murine splenocytes have little ability to generate B(a)P/DNA

adducts, suggesting that hepatic bioactivation is an important mediator of B(a)P-induced immunotoxicity [29]. However, more recent studies by Ladics et al. [58,59] have shown that splenic macrophages can activate B(a)P to the highly reactive 7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydro-B(a)P. Cell separation and reconstitution experiments indicate that macrophages are also the cell type responsible for B(a)P-induced suppression of antibody forming cell responses against T-dependent and T-independent antigens in rodents [6,60]. While splenic and alveolar macrophages showed inducible ethoxyresorufin O-deethylase (EROD) activity, an enzymatic measure of CYP1A1 function, after *in vivo* exposure to the halogenated aromatic hydrocarbon 2,3,7,8-tetrachlorodibenzodioxin (TCDD), isolated lymphocytes and thymocytes do not demonstrate significant metabolic activity [26,27]. Thus, the relative contribution of splenic versus hepatic bioactivation has yet to be determined.

In vitro studies using a murine splenocyte-rat hepatocyte coculture system demonstrate that biotransformation of B(a)P by Phase I enzymes is required in the suppression of T-dependent antibody responses against sheep red blood cells [125]. B(a)P inhibits B cell lymphopoiesis of murine bone marrow cultures and suppresses human T lymphocyte proliferation *in vitro* [15,40,79]. Normal responses were restored when α-naphthoflavone (ANF), a P450-inhibitor, was added in addition to B(a)P in these systems [15,34,40,53]. Direct addition of reactive metabolites, such as the 4,5-epoxide and 7,8 diol, to *in vitro* cultures results in a similar suppression of immune responses. The inability of ANF to restore B(a)P-4,5-epoxide induced suppression of T cell proliferation is further evidence that the immunotoxic effects of B(a)P are caused by reactive metabolites [15].

Nonetheless, in some instances, it appears that PAHs may induce immuno-suppression independent of P450 metabolism. For example, dimethylbenz[a]anthracene (DMBA), which produces many immunologic alterations similar to those seen with B(a)P, can be directly toxic to cultured lymphocytes in the absence of exogenous activation systems [5,14,117]. Addition of ANF to lymphocyte cultures did not modulate DMBA-induced inhibition of concanavalin A stimulated proliferative responses [117]. In these studies, no significant metabolites of DMBA were detected in supernatants from the lymphocyte cultures. However, Ladics et al. [61] have shown that addition of ANF to DMBA-treated splenocyte cultures reestablishes levels of antibody forming cells to that of control cultures. Heidel et al. [42] have recently shown that DMBA can be metabolized by bone marrow stromal cells and that this metabolism is CYP1B1 mediated. These data suggest that DMBA may target multiple immune cell types via different mechanisms and/or metabolic pathways.

4.2. Organic Solvents

Organic solvents are comprised of a few broad chemical classes, including hydrocarbons such as benzene and toluene, halogenated aliphatic hydrocarbons such as carbon tetrachloride and dichloroethane, aliphatic alcohols such as ethanol, and

hydroxyethers such as 2-methoxyethanol. Industrial solvents are frequently mixtures of several compounds. While there is some exposure to the general population via contaminated groundwater or vaporization of commercial solvents and gasolines, the most frequent solvent-associated toxicity occurs from occupational exposure. A number of organic solvents have been examined for their effects on the immune system, and the requirement for their bioactivation to produce immunotoxicity has been established.

Benzene and its metabolites have long been associated with hematologic and immunologic disorders including leukemia in humans. Experimental studies suggest that benzene metabolites, including hydroquinone, catechol and phenol are responsible for its hematotoxicity [49]. Benzene is metabolized by hepatic CYP2E1 primarily to phenol and in turn to hydroquinone and/or catechol [31,37,52,107]. The phenolic metabolites preferentially accumulate in the bone marrow and lymphoid tissues of rodents [3,35,100]. In the bone marrow enzymatic conversion of both phenol and hydroquinone to more reactive binding species such as the semiquinone radical may be P450 independent and involve myeloperoxidases and prostaglandin synthetases [24,48]. The semiquinone radical binds covalently to cellular proteins and forms DNA adducts, disrupting normal cellular functions such as cell division and mitochondrial RNA synthesis [78]. Rapidly proliferating cells, such as lymphoid and myeloid progenitor cells in the bone marrow or clonally expanding lymphocyte subpopulations, are highly sensitive targets for such effects [55,103].

The earliest manifestation of benzene toxicity in exposed workers is a decrease in lymphocyte counts, and a variety of blood disorders, including leukopenia, thrombocytopenia, granulocytopenia and aplastic anemia have been associated with benzene exposure [64,66]. Studies in laboratory animals have demonstrated that treatment with benzene or its metabolites induces myelo- and immunosuppression [112,115]. Benzene and its metabolites appear to be particularly cytotoxic to progenitor cells within the bone marrow, targeting the lymphocyte, monocyte, granulocyte and erythrocyte lineages [65,72,116,118,122]. There is also considerable evidence that benzene metabolites alter the stromal cell microenvironment which supports the differentiation and maturation of these progenitor cells [22,41,55]. *In vivo* exposure to benzene and/or its metabolites inhibits T-dependent antibody responses, B and T cell lymphoproliferative responses and cytotoxic T lymphocyte-mediated tumor cell killing, as well as increases susceptibility to challenge with infectious agents [4,101,102,104].

Several lines of evidence support the hypothesis that reactive metabolites rather than the parent compound, are directly responsible for the observed myelo- and immunotoxicity associated with benzene exposure. Structure activity studies suggest that the polyhydroxy metabolites of benzene have the most immunosuppressive activity, and that benzene and phenol are significantly less toxic to both lymphoid and myeloid cells [8,96,98]. Co-administration of thiol-reactive agents blocks hydroquinone-induced suppression of PHA-stimulated lymphoproliferation and agglutination in rat splenocytes, indicating that oxidation to thiol-reactive quinones may be

a critical step in bioactivation [47,96]. Induction of CYP2E1 in rats by treatment with ethanol enhanced both the metabolism and myelotoxicity of benzene [80]. Finally, inhibition of CYP2E1 activity by administration of propylene glycol partially prevented benzene-induced toxicity in murine peripheral blood lymphocytes and bone marrow cells [119].

4.3. Mycotoxins

Mycotoxins are a diverse group of compounds produced as secondary metabolites from varying species of fungi. Agricultural and laboratory animal studies indicate that in addition to being potent immunosuppressive agents, mycotoxins have genotoxic, embryotoxic, nephrotoxic and carcinogenic effects [94]. Two classes of mycotoxin have been extensively studied for their effects on the immune system. The first class are comprised of the sesquiterpenoids, collectively referred to as tricothecenes, which are produced by *Fusarium* species and the second includes the coumarin derivatives produced by *Aspergillus* and *Penicillium* fungi, such as aflatoxin B_1 (AFB_1) and ochratoxin A. The principal biologically active metabolite of AFB_1 is produced via CYP oxidation of the 8,9 double bond, forming a highly reactive AFB_1-8,9-epoxide which can bind to nucleic acids and cellular proteins, as well as several less toxic hydroxylated metabolites [74]. CYP3A4 appears to be primarily involved in the detoxification of AFB_1, whereas CYP1A2 is the primary agent involved in AFB_1 bioactivation [23]. However, the specific CYP isozymes responsible for the activation of AFB_1 are species-specific and appear to be dependent upon substrate concentration, which may account for some of the reported variability in immunotoxicity [2,21,82].

In vitro studies using a mouse splenocyte/rat hepatocyte coculture system demonstrate that activation of AFB_1 by Phase I enzymes plays a significant role in suppression of T-dependent antibody responses against sheep red blood cells [125]. The numbers of antibody forming cells was significantly reduced in the presence of AFB_1 in the cocultures. However, in the absence of the hepatocyte activation system, antibody responses were at control levels in AFB_1-exposed splenocytes. This is not surprising, as rodent lymphocytes have shown minimal metabolic activity [27,59]. Suppression of phagocytic activity and alterations in cell morphology in turkey and chicken macrophages cultured in the presence of AFB_1 were similarly dependent on metabolic activation of the parent compound, and the adverse effects were reversed in the presence of the P450 inhibitor, piperonyl butoxide [85,86]. These results suggest that macrophages from avian species may be unable to activate AFB_1 to reactive intermediates. This is supported by the studies of Lorr et al. [68] who demonstrated that only minimal EROD activity could be found in peritoneal macrophages in chickens induced with tetrachlorobiphenyl. In contrast, AFB_1 has suppressive effects on rodent and human macrophages in the absence of outside activation systems [12,13]. When added to cultures of rat peritoneal cells, AFB_1 alone, as well as its metabolites AFB_2, AFG_1, AFG_2, and AFM_1

depressed phagocytic capacity, cytotoxicity and secretion of reactive oxygen species. This suggests that, at least in some species, extrahepatic metabolism may play a role in AFB1-induced toxicity to macrophages. A number of studies have demonstrated that rodent and human macrophages possess metabolic activity [26,60,95].

In vivo, differences in constitutive expression and induction of CYP isozymes may have a significant influence on toxicity by affecting the equilibrium between formation of reactive and detoxified primary metabolites of AFB_1 [82]. This may be of critical importance with regard to toxicity in domestic animals, where immunosuppressive effects from ingesting mycotoxin-contaminated feed have been observed at levels where no overt toxicity is seen [99]. *In vivo* studies in rodents, poultry and livestock suggest that cell-mediated immune parameters are particularly sensitive to insult by AFB_1 and that the innate immune system may also be a susceptible target [93,111]. Respiratory exposure to AFB_1 induces similar systemic and localized immunosuppressive effects in both rats and mice [51].

4.4. Anti-neoplastic agents

The association between the clinical use of cancer chemotherapeutics and immunosuppressive drugs, such as those used in organ transplant recipients, and increased incidence of infectious and neoplastic disease has been well established [19,92]. Although not metabolized by P450s, anti-neoplastic agents such as azathioprine, methotrexate and 5-fluorouracil frequently exhibit immunosuppressive activity, due to their direct toxicity to the bone marrow and induction of DNA damage in rapidly proliferating cells [19,124]. Cyclophosphamide is a prototype cancer chemotherapeutic, and its immunomodulatory effects have been studied extensively in humans and in experimental animals [18]. Metabolic activation of cyclophosphamide by cytochrome P450 leads to the formation of the intermediates 4-OH-cyclophosphamide and aldophosphamide. Aldophosphamide undergoes a spontaneous β-elimination reaction resulting in the formation of two cytotoxic metabolites, phosphoramide mustard and acrolein [9,10]. Aldehyde dehydrogenases detoxify cyclophosphamide through the conversion of aldophosphamide to carboxyphosphamide [43] and have been shown to reduce the myelotoxicity of cyclophosphamide in rodent bone marrow cells and to mediate drug tolerance in resistant tumor cell lines [105,106]. Many toxic effects of cyclophosphamide are thought to be the result of the alkylating activity of the phosphoramide mustard, leading to inhibition of DNA replication, although acrolein can also produce cytotoxicity through covalent binding to sulfhydryl groups of cellular proteins [73,109].

In humans, cyclophosphamide appears to preferentially target B lymphocytes at therapeutic doses, though higher doses proved equally cytotoxic to B and T cells [11,45]. Similar findings have been demonstrated in rodent and avian species [20,67,108,114,123]. Studies in the chicken suggest that bioactivation in extrahepatic tissues is not a factor in the selective toxicity to B lymphocytes in these animals, as there were no tissue-specific differences in cyclophosphamide metabolism in T

versus B lymphocytes [76]. Therefore metabolism may be primarily hepatic. A number of studies have demonstrated that specific T-suppressor cell populations may be preferentially sensitive to the effects of cyclophosphamide and its metabolites [77,110,120]. This may explain why in some instances, especially at low doses, treatment with cyclophosphamide can potentiate immune responses, such as resistance to tumor challenge [69].

Mechanistic studies to identify particular metabolites of cyclophosphamide which are responsible for the immunosuppressive and cell-specific effects have met with mixed results. To determine whether the oxazaphosphorine moiety is necessary to suppress immune cells, Smith and Sladek [114] examined the differential effects of 4-hydroxycyclophosphamide/aldophosphamide and phosphoramide mustard on mitogen-induced proliferative responses in murine B- and T-lymphocytes. These studies indicated that 4-hydroxycyclophosphamide was the principal mediator of the selective immunotoxicity. In contrast, Wilmer et al. [123] found that isophosphamide, 4-methylcyclophosphamide and the phosphoramide mustard were preferentially toxic to avian B-lymphocytes. Direct addition of acrolein indicates that it is a more potent inhibitor of suppressor T cell activity and *in vitro* antibody responses than is phosphoramide mustard [54]. These authors suggest that this inhibition may be mediated via the binding of acrolein to sulfhydryl-containing molecules unique to suppressor T cells. As cyclophosphamide targets distinct immune cell populations, the difficulty in determining the critical metabolites responsible for its toxic effects is not surprising and interspecies variation in metabolism may account for some of the discrepancies.

5. Summary

The elucidation of the metabolic pathways for enzymatic biotransformation of xenobiotics and immunotoxicity studies are areas which do not frequently overlap. However, it can be seen from the above examples that knowledge of metabolism and identification of the ultimate toxic species is critical in understanding the mechanisms of immunotoxicity and the target cell populations for a wide variety of xenobiotics. Furthermore, an awareness of how these two processes interrelate are essential to conduct risk assessment in immunotoxicology taking into account likely metabolites and polymorphisms in humans of Phase I and II enzyme systems. Such concerns also exist in the design of therapeutics used to treat HIV infections, transplant rejection and other diseases that affect the immune system.

Acknowledgments

The authors would like to thank Drs. Joyce Goldstein and William Baldwin for their thoughtful comments and critical evaluation of this work.

References

[1] D.L. Alexander, S. Eltom, P.M. Fernandez-Salguero, F. Gonzalez and C.R. Jefcoate, Ah receptor dependent expression of CYP1B1 in mouse embryo cells from normal and Ah receptor deficient mice, *The Toxicologist* **36** (1997), 132 (Abstract).

[2] R.M. Almeida, B. Correa, J.G. Xavier, M.A. Mallozzi, W. Gambale and C.R. Paula, Acute effect of aflatoxin B1 on different inbred mouse strains II, *Mycopathologia* **133** (1996), 23–29.

[3] L.S. Andrews, W.E. Lee, C.M. Witmer, J.J. Kocsis and R. Snyder, Effects of toluene on the metabolism, disposition and hemopoietic toxicity of [^{3}H]benzene, *Biochem. Pharmacol.* **26** (1977), 293–300.

[4] K. Aoyama, Effects of benzene inhalation on lymphocyte subpopulations and immune response in mice, *Toxicol. Appl. Pharmacol.* **85** (1986), 92–101.

[5] R.H. Blanton, M. Lyte, M.J. Myers and P.H. Bick, Immunomodulation by polyaromatic hydrocarbons in mice and murine cells, *Cancer. Res.* **46** (1986), 2735–2739

[6] R.H. Blanton, M.J. Myers and P.H. Bick, Modulation of immunocompetent cell populations by benzo[a]pyrene, *Toxicol. Appl. Pharmacol.* **93** (1988), 267–274.

[7] C. Bolognesi, L. Rossi, O. Barbieri and L. Santi, Benzo(a)pyrene-induced DNA damage in mouse fetal tissues, *Carcinogenesis* **6** (1985), 1091–1095.

[8] S.C. Cheung, D.E. Nerland and G. Sonnerfeld, Inhibition of interferon gamma production by benzene and benzene metabolites, *J. Natl. Cancer Inst.* **80** (1988), 1069–1072.

[9] M. Colvin and J. Hilton, Pharmacology of cyclophosphamide and metabolites, *Cancer Treat. Rep.* **65** (S3) (1981), 89–95.

[10] T.A. Connors, P.J. Cox, P.B. Farmer, A.B. Foster and M. Jarman, Some studies on the active intermediates formed in the microsomal metabolism of cyclophosphamide and isophosphamide, *Biochem. Pharmacol.* **23** (1974), 115–129.

[11] T.T. Cupps, L.C. Edgar and A.S. Fauci, Suppression of human B lymphocyte function by cyclophosphamide, *J. Immunol.* **128** (1982), 2453–2457.

[12] V. Cusumano, G.B. Costa and S. Seminara, Effect of aflatoxins on rat peritoneal macrophages, *Appl. Environ. Microbiol.* **56** (1990), 3482– 3484.

[13] V. Cusumano, F. Rossano, R.A. Merendino, A. Arena, G.B. Costa, G. Mancuso, A. Baroni and E. Losi, Immunobiological activities of mold products: functional impairment of human monocytes exposed to aflatoxin B1, *Res. Microbiol.* **147** (1996), 385–391.

[14] D.R. Davila, D.P. Davis, K. Campbell, J.C. Cambier, L.A. Zigmond and S.W. Burchiel, Role of alterations in Ca (2+)- associated signaling pathways in the immunotoxicity of polycyclic aromatic hydrocarbons, *J. Toxicol. Environ. Health.* **45** (1995), 101–126.

[15] D.R. Davila, D.L. Romero and S.W. Burchiel, Human T cells are highly sensitive to suppression of mitogenesis by polycyclic aromatic hydrocarbons and this effect is differentially reversed by alpha-napthoflavone, *Toxicol. Appl. Pharmacol.* **139** (1996), 333–341.

[16] D.A. Davis and S.W. Burchiel, Inhibition of calcium-dependent pathways of B-cell activation by DMBA, *Toxicol. Appl. Pharmacol.* **116** (1992), 202–208.

[17] J.H. Dean, M.I. Luster, A.E. Munson and I. Kimber, eds., *Immunotoxicology and Immunopharmacology, 2nd edition*, Raven Press, New York, 1994.

[18] J.G. Descotes and T. Vial, Cytoreductive drugs. In: *Immunotoxicology and Immunopharmacology, 2nd edition*, M.I. Luster, J.H. Dean, A.E. Munson and I. Kimber, eds., Raven Press, New York, 1994, pp. 293–301.

[19] M.J. Ehrke, Effects of cancer therapy on host response and immunobiology, *Curr. Opin. Oncol.* **3** (1991), 1070–1077.

[20] J. Eskola and P. Toivanen, Effect of in *ovo* treatment with cyclophosphamide on lymphoid system in chicken, *Cell. Immunol.* **13** (1974), 459–471.

[21] L.M. Forrester, G.E. Neal, D.J. Judah, M.J. Glancey and C.R. Wolf, Evidence for involvement of multiple forms of cytochrome P-450 in aflatoxin B1 metabolism in human liver, *Proc. Natl. Acad. Sci.* **87** (1990), 8306–8310.

[22] K.W. Gaido and D. Wierda, Modulation of stromal cell function in DBA/2J and B6C3F1 mice exposed to benzene or phenol, *Toxicol. Appl. Pharmacol.* **81** (1985), 469–475.

[23] E.P. Gallagher, L.C. Wienkers, P.L. Stapleton, K.L. Kunze and D.L. Eaton, Role of human microsomal and human complementary DNA-expressed cytochromes P4501A2 and P4503A4 in the bioactivation of aflatoxin B1, *Cancer Res.* **54** (1994), 101–108.

[24] L.G. Ganousis, D. Goon, T. Zyglewska, K. Wu and D. Ross, Cell-specific metabolism in mouse bone marrow stroma: Studies of activation and detoxification of benzene metabolites, *Mol. Pharmacol.* **42** (1992), 1118–1125.

[25] H.V. Gelboin, Carcinogens, enzyme induction, and gene action, *Adv. Cancer Res.* **10** (1967), 1–81.

[26] D.R. Germolec, N.H. Adams and M.I. Luster, A comparative assessment of metabolic enzyme levels in macrophage subpopulations in the F344 rat, *Biochem. Pharmacol.* **50** (1995), 1495–1504.

[27] D.R. Germolec, E.C. Henry, R. Maronpot, J.F. Foley, N.H. Adams, T.A. Gasiewicz and M.I. Luster, Induction of CYP1A1 and ALDH-3 in lymphoid tissues from Fisher 344 rats exposed to 2,3,7,8-tetrachlorodibenzodioxin (TCDD), *Toxicol. Appl. Pharmacol.* **137** (1996), 57–66.

[28] G.L. Ginsburg and T.B. Atherholt, Transport of DNA-adducting metabolites in mouse serum following benzo[a]pyrene administration, *Carcinogenesis.* **10** (1989), 673–679.

[29] G.L. Ginsburg, T.B. Atherholt and G.H. Butler, Benzo[a]pyrene-induced immunotoxicity: comparison to DNA adduct formation *in vivo*, in cultured splenocytes, and in microsomal systems, *J. Toxicol. Environ. Health.* **28** (1989), 205–220.

[30] J.A. Goldstein and M.B. Faletto, Advances in Mechanism of Activation and Deactivation of Environmental Chemicals, *Envrion. Health Perspect.* **100** (1993), 169–176.

[31] L.M. Gonasun, C. Witmer, J.J. Kocsis and R. Snyder, Benzene metabolism in mouse liver microsomes, *Toxicol. Appl. Pharmacol.* **26** (1970), 398–406.

[32] F.J. Gonzalez and Y.-H. Lee, Constitutive expression of hepatic cytochrome P450 genes, *FASEB J.* **10** (1996), 1112–1117.

[33] F.J. Gonzalez and D.W. Nebert, Evolution of the P450 gene superfamily: animal-plant warfare, molecular drive and human genetic differences in drug oxidation, *Trends Genet.* **6** (1990), 182–186.

[34] F.M. Goujon, D.W. Nebert and J.E. Gielen, Genetic expression of aryl hydrocarbon hydroxylase induction. IV. Interaction of various compounds with different forms of cytochrome P-450 and the effect on benzo[a]pyrene metabolism *in vitro*, *Mol. Pharmacol.* **8** (1972), 667–680.

[35] W.F. Greenlee, E.A. Gross and R.D. Irons, Relationship between benzene toxicity and the disposition of ^{14}C-labeled benzene metabolites in the rat, *Chem. Biol. Interact.* **33** (1981), 285–299.

[36] F.P. Guengerich, Enzymatic oxidation of xenobiotic chemicals, *Crit. Rev. Biochem. Mol. Biol.* **25** (1990), 97–153.

[37] F.P. Guengerich, D.-H. Kim and M. Iwasaki, Role of human cytochrome P-450 IIE1 in the oxidation of many low molecular weight cancer suspects, *Chem. Res. Toxicol.* **4** (1991), 168–179.

[38] J.R. Halpert, F.P. Guengerich, J.R. Bend and M.A. Correia, Selective Inhibitors of Cytochromes P450, *Toxicol. Appl. Pharmacol.* **125** (1994), 163–175.

[39] J.W. Hamilton and S.E. Bloom, Correlation between induction of xenobiotic metabolism and DNA damage from chemical carcinogens in the chick embryo *in vivo*, *Carcinogenesis* **7** (1986), 1101–1106.

[40] J.A. Hardin, F. Hinoshita and D.H. Sherr, Mechanisms by which benzo[a]pyrene, an environmental carcinogen, suppresses B cell lymphopoiesis, *Toxicol. Appl. Pharmacol.* **117** (1992), 155–164.

[41] K. Harigaya, M.E. Miller, E.P. Cronkite and R.T. Drew, The detection of *in vivo* hematotoxicity of benzene by *in vitro* liquid bone marrow cultures, *Toxicol. Appl. Pharmacol.* **60** (1981), 346–353.

[42] S.M. Heidel, C.J. Czuprynski and C.R. Jefcoate, Metabolism of DMBA by CYP1B1 in Bone Marrow Stromal Cells is influenced by the Ah receptor, *The Toxicologist* **36** (1997), 197 (Abstract).

[43] J.H. Hipkens, R.F. Struck and H.L. Gurtoo, Role of aldehyde dehydrogenase in the metabolism dependent biological activity of cyclophosphamide, *Cancer Res.* **41** (1981), 3571–3583.

[44] S.D. Holladay and B.J. Smith, Benzo[a]pyrene-induced alterations in total immune cell number and cell-surface antigen expression in the thymus, spleen and bone marrow of B6C3F1 mice, *Vet. Human Toxicol.* **37** (1995), 99–104.

[45] E.R. Hurd and V.J. Giuliano, The effect of cyclophosphamide on B and T lymphocytes in patients with connective tissue disease, *Arthritis Rheum.* **18** (1975), 67–75.

[46] C. Ioannides and D.V. Parke, Induction of cytochrome P4501 as an indicator of potential chemical carcinogenesis, *Drug. Metab. Rev.* **25** (1993), 485–501.

[47] R.D. Irons and R.W. Pfeiffer, Benzene metabolite: Evidence for an epigenetic mechanism of toxicity. In: *Proceedings of the Genotoxic Effects of Airborne Agents*, Brookhaven National Laboratory, New York, 1982, pp. 241–256.

[48] R.D. Irons and T. Sawahata, Phenols, catechols, and quinones. In: *Bioactivation of Foreign Compounds*, M.W. Anders, ed., Academic Press, New York, 1985, pp. 259–281.

[49] R.D. Irons, W.F. Greenlee, D. Wierda and J.S. Bus, Relationship between benzene metabolism and toxicity: A proposed mechanism for the formation of reactive intermediates. In: *Biological Reactive Intermediates-II*, R. Snyder, D.V. Parke, J.J. Kocsis, D.J. Jollow, C.G. Gibson and C.M. Witmer, eds., Plenum Press, New York, 1982, pp. 229–243.

[50] G. Jacob, K. Byth and G.C. Farrell, Age but not gender selectively affects expression of individual cytochrome P450 proteins in human liver, *Biochem. Pharmacol.* **50** (1995), 727–730.

[51] G.J. Jakab, R.R. Hmieleski, A. Zarba, D.R. Hemenway and J.D. Groopman, Respiratory aflatoxicosis: Suppression of pulmonary and systemic host defenses in rats and mice, *Toxicol. Appl. Pharmacol.* **125** (1994), 198–205.

[52] I. Johansson and M. Ingelman-Sundberg, Benzene metabolism by ethanol-, acetone-, and benzene-inducible cytochrome P-450 (IIE1) in rat and rabbit liver microsomes, *Cancer Res.* **48** (1988), 5387– 5390.

[53] T.T. Kawabata and K.L. White, Suppression of the *in vitro* humoral immune response of mouse splenocytes by benzo[a]pyrene metabolites and inhibition of benzo[a]pyrene-induced immunosuppression by a-napthoflavone, *Cancer Res.* **47** (1987), 2317–2322.

[54] T.T. Kawabata and K.L. White, Enhancement of *in vivo* and *in vitro* murine immune responses by the cyclophosphamide metabolite acrolein, *Cancer Res.* **48** (1988), 41–45.

[55] A.G. King, K.S. Landreth and D. Wierda, Bone marrow stromal cell regulation of B-lymphopoiesis. II. Mechanisms of hydroquinone inhibition of pre-B cell maturation, *J. Pharmacol. Exp. Therap.* **250** (1989), 582–590.

[56] K.L. Kunze, B.L.K. Mangold, C. Wheeler, H.S. Beilan and P.R. Ortiz de Montellano, The cytochrome P-450 active site, *J. Biol. Chem.* **258** (1983), 4202–4207.

[57] G.S. Ladics and K.L. White, Jr., Immunotoxicity of polyaromatic hydrocarbons, In: *Experimental Immunotoxicology*, R.J. Smialowicz and M.P. Holsapple, eds., CRC Press, New York, 1996, pp. 331–350.

[58] G.S. Ladics, T.T. Kawabata, A.E Munson and K.L. White, Jr., Generation of 7,8-Dihydroxy-9,10-epoxy-7,8,9,10-tetrahydro-benzo[a]pyrene by Murine Splenic Macrophages, *Toxicol. Appl. Pharmacol.* **115** (1992), 72–79.

[59] G.S. Ladics, T.T. Kawabata, A.E. Munson and K.L. White, Jr., Metabolism of benzo[a]pyrene by murine splenic cell types, *Toxicol. Appl. Pharmacol.* **116** (1992), 248–257.

[60] G.S. Ladics, T.T. Kawabata, A.E. Munson and K.L. White, Jr., Evaluation of murine splenic cell type metabolism of benzo[a]pyrene and functionality *in vitro* following repeated *in vivo* exposure to benzo[a]pyrene, *Toxicol. Appl. Pharmacol.* **116** (1992), 258–266.

[61] G.S. Ladics, T.T. Kawabata and K.L. White, Jr., Suppression of the *in vitro* humoral immune response of mouse splenocytes by 7, 12-dimethylbenz[a]anthracene metabolites and inhibition of immunosuppression by a-napthoflavone, *Toxicol. Appl. Pharmacol.* **110** (1991), 31–44.

[62] A. Lampen, U. Christians, A. Bader, I. Hackbarth and K.-F. Sewing, Interindividual variability of Ciclosporin metabolism in the small intestine, *Pharmacology* **52** (1996), 159–168.

[63] M. Larsen, W.G.R. Angus, P. Brake, S. Eltom and C.R. Jefcoate, CYP1B1 represents the major PAH-responsive P450 cytochrome constitutively expresses in normal primary HMEC, *The Toxicologist* **36** (1997), 24 (Abstract).

[64] S. Laskin and B.D. Goldstein, Benzene toxicity, *J. Toxicol. Environ. Health Suppl.* **2** (1977), 1–313.

[65] E.W. Lee, J.J. Kocsis and R. Snyder, Acute of benzene on ^{59}Fe incorporation into circulating erythrocytes, *Toxicol. Appl. Pharmacol.* **27** (1974), 431–436.

[66] S.D. Lee, M. Dourson, D. Murkerjee, J.F. Stara and T. Kawecki, Assessment of benzene health effects in ambient water, *Adv. Mod. Environ. Toxicol.* **4** (1983), 91–125.

[67] T.J. Linna, D. Frommel and R.A. Good, Effects of early cyclophosphamide treatment on the development of lymphoid organs and immunological functions in the chicken. *Int. Arch. Allergy Appl. Immunol.* **42** (1972), 20–39.

[68] N.A. Lorr, K.A. Golemboski, R.A. Hemendinger, R.R. Dietert and S.E. Bloom, Distribution and inducibility of a P450I activity in cellular components of the avian immune system. *Arch. Toxicol.* **60** (1992), 560–566.

[69] M.I. Luster, C. Portier, D.G. Pait, G.J. Rosenthal, D.R. Germolec, E. Corsini, B.L. Blaylock, P. Pollock, Y. Kouchi, W. Craig, K.L. White, Jr., A.E. Munson and C.E. Comment, Risk assessment in immunotoxicology. II. Relationships between immune and host resistance test, *Fundam. Appl. Toxicol.* **21** (1993), 71–82.

[70] M.I. Luster, D. Wierda, and G.J. Rosenthal, Environmentally related disorders of the hematologic and immune systems, *Med. Clin. of North Am.* **74** (1990), 425–439.

[71] M.L. Lyte, R.H. Blanton, M.J. Myers and P.H. Bick, Effect of *in vivo* administration of the carcinogen benzo[a]pyrene on interleukin-2 and interleukin-3 production, *Int. J. Immunopharmacol.* **9** (1987), 307–312.

[72] L. MacEachern, R. Snyder and D.L. Laskin, Alterations inn the morphology and functional activity of bone marrow phagocytes following benzene treatment of mice. *Toxicol. Appl. Pharmacol.* **117** (1992), 147–154.

[73] A.J. Marinello, S.K. Bansal, B. Paul, P.L. Koser, J. Love, R.F. Struck and H.L. Gurtoo, Metabolism and binding of cyclophosphamide and its metabolite acrolein to rat hepatic microsomal cytochrome P-450, *Cancer Res.* **44** (1984), 4615–4621.

[74] M. McLean and M.F. Dutton, Cellular interactions and metabolism of aflatoxin: An update, *Pharmac. Ther.* **65** (1995), 163–192.

[75] U.A. Meyer, F. J. Gonzalez, F.P. Guengerich, M.E. McManus and K.-I. Okuda, Human cytochrome P450: Regulation and functional variability. In: *Pharmacological Sciences: Perspectives for Research and Therapy in the late 1990s*, A.C. Cuello and B. Collier, eds.,. Birkhauser Verlag, Basel, 1995, pp. 153–159.

[76] R.R. Misra, N.A. Lorr and S.E. Bloom, Cyclophosphamide metabolism in the primary immune organs of the chick: assays of drug activation, P450 expression, and aldehyde dehydrogenase, *Arch. Toxicol.* **65** (1991), 32–38.

[77] A. Mitsuoka, M. Baba and S. Morikawa, Enhancement of delayed hypersensitivity by depletion of suppressor T cells with cyclophosphamide in mice, *Nature* 262 (1976), 77–78.

[78] P. Moldeus, D. Ross and R. Larsson, Inter-relationships between xenobiotic metabolism and lipid biochemistry, *Biochem. Soc. Trans.* **13** (1985), 847–850.

[79] S.P. Mudzinski, Effects of benzo[a]pyrene on concanavalin A-stimulated human peripheral blood mononuclear cells *in vitro*: Inhibition of proliferation but no effect on parameters related to the G1 phase of the cell cycle, *Toxicol. Appl. Pharmacol.* **199** (1993), 166–174.

[80] T. Nakajima, S. Okuyama, I. Yonekura and A. Sato, Effects of ethanol and phenobarbital administration on the metabolism and toxicity of benzene, *Chem.-Biol. Interactions* **55** (1985), 23–38.

[81] National Research Council: Subcommittee on Immunotoxicology, *Biologic Markers in Immunotoxicology*. National Academy Press, Washington, DC, 1992.

[82] G.E. Neal, Genetic implications in the metabolism and toxicity of mycotoxins. *Toxicol. Lett.* **82/83** (1995), 861–867.

[83] D.W. Nebert, Multiple forms of inducible drug-metabolizing enzymes: A reasonable mechanism by which any organism can cope with adversity, *Mol. Cell. Biochem.* **27** (1979), 27–46.

[84] D.W. Nebert, H.J. Eisen, M. Negishi, M.A. Lang and L.M. Hjelmeland, Genetic differences in susceptibility to chemically induced myelotoxicity and leukemia, *Environ. Health. Perspect.* **39** (1981), 11–22.

[85] D.L. Neldon-Ortiz and M.A. Qureshi, Direct and microsomal activated aflatoxin B1 exposure and its effects on turkey peritoneal macrophage functions *in vitro*, *Toxicol. Appl. Pharmacol.* **109** (1991), 432–442.

[86] D.L. Neldon-Ortiz and M.A. Qureshi, The effects of direct and microsomal activated aflatoxin B1 on chicken peritoneal macrophages *in vitro*, *Vet. Immunol. Immunopath.* **31** (1992), 61–76.

[87] D.R. Nelson, L. Koymans, T. Kamataki, J.J. Stegeman, R. Feyereisen, D.J. Waxman, M.R. Waterman, O. Gotoh, M.J. Coon, R.W. Estabrook, I.C. Gunsalus and D.W. Nebert, P450 superfamily: Update on new sequences, gene mapping, accession numbers and nomenclature, *Pharmacogenetics* **6** (1996), 1–42.

[88] A. Paine, Heterogeneity of cytochrome P450 and its toxicological significance, *Human Exp. Toxicol.* **14** (1995), 1–7.

[89] M. Pallardy, Z. Mishal, H. Lebrec and C. Bohoun, Immune modification due to chemical interference with transmembrane signaling: applications to polycyclic aromatic hydrocarbons, *Int. J. Immunopharmacol.* **14** (1992), 377–382.

[90] B.K. Park, P. Munir and N.P. Kitteringham, The role of cytochrome P450 enzymes in hepatic and extrahepatic human drug toxicity, *Pharmac. Ther.* **68** (1995), 385–424.

[91] A. Parkinson, Biotransformation of Xenobiotics. In: *Casarett & Doull's Toxicology: The Basic Science of Poisons; Fifth Edition*, C.D. Klaassen, ed., McGraw-Hill, New York, 1996, pp. 113–186.

[92] I. Penn, Neoplastic consequences of immunosuppression. In: *Immunotoxicology and Immunopharmacology*, J.H. Dean, M.I. Luster, A.E. Munson and H. Amos, eds., Raven Press, New York, 1985, pp. 79–90.

[93] J.J. Pestka and G.S. Bondy, Mycotoxin-induced immune modulation. In: *Immunotoxicology and Immunopharmacology, 2nd edition*, M.I. Luster, J.H. Dean, A.E. Munson and I. Kimber, eds., Raven Press, New York, 1994, pp. 293–301.

[94] J.J. Pestka and W.L. Casale, Naturally occurring fungal toxins. In: *Food contamination from environmental sources*, M.S. Simmons and J Nriagu, eds., Wiley, New York, 1990, pp. 613–638.

[95] T.C. Peterson, Drug-metabolizing enzymes in rat, mouse, pig and human macrophages and the effect of phagocytic activation, *Biochem. Pharmacol.* **36** (1987), 3911–3916.

[96] R.W. Pfeiffer and R. D. Irons, Inhibition of lectin-stimulated lymphocyte agglutination and mitogenesis by hydroquinone: Reactivity with intracellular sulfhydryl groups, *Exp. Mol. Pathol.* **35** (1981), 189–198.

[97] T.D. Porter and M.J. Coon, Cytochrome P-450: Multiplicity of isoforms, substrates and catalytic and regulatory mechanisms, *J. Biol. Chem.* **266** (1991), 13469–13472.

[98] G.B. Post, R. Snyder and G.F. Kalf, Inhibition of RNA synthesis and interleukin-2 production in lymphocytes by benzene and its metabolites, hydroquinone and p-benzoquinone. *Toxicol. Lett.* **29** (1985), 161–167.

[99] J.J. Richard, J.R. Thurston and A.C. Pier, Effects of mycotoxins on immunity. In: *Toxins: Animal, plant and microbial*, P. Rosenberg, ed., Pergamon Press, New York, 1978, pp. 801–818.

[100] D.E. Rickert, T.S. Baker, J.S. Bus, C.S. Barrow and R.D. Irons, Benzene disposition in the rat after exposure by inhalation, *Toxicol. Appl. Pharmacol.* **49** (1979), 417–423.

[101] G.J. Rosenthal, G.J. and C.A. Snyder, Modulation of the immune response to Listeria monocytogenes by benzene inhalation, *Toxicol. Appl. Pharmacol.* **80** (1985), 502–510.

[102] G.J. Rosenthal, G.J. and C.A. Snyder, Inhaled benzene reduces aspects of cell-mediated tumor surveillance in mice, *Toxicol. Appl. Pharmacol.* **88** (1987), 35–43.

[103] M. Rozen and C.A. Snyder, Protracted exposure of C57Bl/6 mice to 300 ppm benzene depresses B- and T-lymphocyte numbers and mitogen responses. Evidence for thymic and bone marrow proliferation in response to the exposures, *Toxicology* **20** (1985), 343–349.

[104] M.G. Rozen, C.A. Snyder and R.E. Albert, Depressions in B-and T-lymphocyte mitogen-induced blastogenesis in mice exposed to low concentrations of benzene, *Toxicol. Lett.* **20** (1984), 343–349.

[105] J.E. Russo and J. Hilton, Characterization of cytosolic aldehyde dehydrogenase from cyclophosphamide resistant 1210 cells, *Cancer Res.* **48** (1988), 2963–2968.

[106] E.A. Sahovic, M. Colvin, J. Hilton and M. Ogawa, Role for aldehyde dehydrogenase in survival of progenitors for murine blast cell colonies after treatment with 4-hydroperoxy-cyclophosphamide *in vitro*, *Cancer Res.* **48** (1988), 1223–1226.

[107] M.J. Seaton, P.M. Schlosser, J.A. Bond and M.A. Medinsky, Benzene metabolism by human liver microsomes in relation to cytochrome P450 2E1 activity, *Carcinogenesis* **15** (1994), 1799–1806.

[108] F.L. Shand, The capacity of microsomally-activated cyclophosphamide to induce immunosuppression *in vitro*, *Immunology* **35** (1978), 1017–1025.

[109] F.L. Shand, The immunopharmacology of cyclophosphamide, *Int. J. Immunopharmacol.* 1 (1979), 165–171.

[110] F.L. Shand and F.Y. Liew, Differential sensitivity to cyclophosphamide of helper T cells for humoral responses and suppressor T cells for delayed-type hypersensitivity, *Eur. J. Immunol.* **10** (1980), 480–483.

[111] R.P. Sharma, Immunotoxicity of mycotoxins. *J. Dairy Sci.* **76** (1993), 892–897.

[112] R.J. Smialowicz, The Immunotoxicity of Organic Solvents, In: *Experimental Immunotoxicology*, R.J. Smialowicz and M.P. Holsapple, eds., CRC Press, New York, 1996, pp. 307–330.

[113] R.J. Smialowicz and Holsapple, M.P., *Experimental Toxicology*, CRC Press, New York, 1996.

[114] P.C. Smith and N.E. Sladek, Sensitivity of murine B- and T-lymphocytes to oxazaphosphorine and nonoxazaphosphorine nitrogen mustards, *Biochem. Pharmacol.* **34** (1985), 3459–3463.

[115] C.A. Snyder, Organic Solvents. In: *Immunotoxicology and Immunopharmacology, 2nd edition*, M.I. Luster, J.H. Dean, A.E. Munson and I. Kimber, eds., Raven Press, New York, 1994, pp. 183–190.

[116] D.J. Thomas, M.J. Reasor and D. Wierda, Macrophage myelopoiesis is altered by exposure to the benzene metabolite hydroquinone, *Toxicol. Appl. Pharmacol.* **97** (1989), 440–453.

[117] L.M. Thurmond, R.V. House, L.D. Lauer and J.H. Dean, Suppression of splenic lymphocyte function by 7,12-dimethylbenz[a]anthracene (DMBA) *in vitro*, *Toxicol. Appl. Pharmacol.* **93** (1988), 369–377.

[118] A. Tunek, T. Olofsson and M. Berlin, Toxic effects of benzene and benzene metabolites on granulopoietic stem cells and bone marrow cellularity in mice, *Toxicol. Appl. Pharmacol.* **59** (1981), 149– 156.

[119] J. Tuo, S. Loft, M.S. Thomsen and H.E. Poulsen, Benzene-induced genotoxicity in mice *in vivo* detected by the alkaline comet assay: reduction by CYP2E1 inhibition, *Mutation Res.* **368** (1996), 213–219.

[120] J.L. Turk and D. Parker, The effect of cyclophosphamide on the immune response, *J. Immunopharmacol.* **1** (1979), 127–137.

[121] K.L. White, Jr., H.H. Lysy and M.J. Holsapple, Immunosuppression by polycyclic aromatic hydrocarbons: a structure-activity relationship in B6C3F1 and DBA/2 mice, *Immunopharmacology.* **9** (1985), 155–164.

[122] D. Wierda and R. Irons, Hydroquinone and catechol reduce the frequency of progenitor B lymphocytes in mouse spleen and bone marrow, *Immunopharmacology* **4** (1982), 41–54.
[123] J.L. Wilmer, O.M. Colvin and S.E. Bloom, Cytogenetic mechanisms in the selective toxicity of cyclophosphamide analogs and metabolites towards avian embryonic B lymphocytes *in vivo*, *Mutation Res.* **268** (1992), 115–130.
[124] A. Winkelstein, Immune suppression resulting from various cytotoxic agents, *Clin. Immunol. Allergy* **4** (1984), 295–315.
[125] K.H. Yang, B.S. Kim, A.E. Munson and M.P. Holsapple, Immunosuppression induced by chemicals requiring metabolic activation in mixed cultures of rat hepatocytes and murine splenocytes, *Toxicol. Appl. Pharmacol.* **83** (1986), 420–429.